

## AMENDMENTS

### Amendments to the Claims

1-3. (Canceled)

4. (Previously presented) A botulinum toxin serotype A (BoNT/A) substrate, comprising:

(a) a donor fluorophore;

(b) an acceptor fluorophore having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(c) a BoNT/A recognition sequence comprising a cleavage site, wherein said cleavage site intervenes between said donor fluorophore and said acceptor fluorophore;

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor fluorophore.

5. (Currently amended) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate comprises at least six consecutive residues of SNAP-25, or a peptidomimetic thereof, said six consecutive residues comprising Gln-Arg, or a peptidomimetic thereof.

6. (Currently amended) The BoNT/A substrate of claim 5, wherein said BoNT/A substrate comprises at least six consecutive residues of a human SNAP-25, or a peptidomimetic thereof, said six consecutive residues comprising Gln<sub>197</sub>-Arg<sub>198</sub>, or a peptidomimetic thereof.

7. (Previously presented) The BoNT/A substrate of claim 6, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 27, or a peptidomimetic thereof.

8. (Previously presented) The BoNT/A substrate of claim 6, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 29, or a peptidomimetic thereof.

9-44. (Canceled)

45. (Previously presented) The BoNT/A substrate of claim 4, wherein said substrate can be cleaved with an activity of at least 1 nanomole/minute/milligram toxin.

46. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate can be cleaved with an activity of at least 20 nanomole/minute/milligram toxin.

47. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate can be cleaved with an activity of at least 50 nanomole/minute/milligram toxin.

48. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate can be cleaved with an activity of at least 100 nanomole/minute/milligram toxin.

49. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate can be cleaved with an activity of at least 150 nanomole/minute/milligram toxin.

50. (Canceled)

51. (Previously presented) The BoNT/A substrate of claim 4, wherein said acceptor fluorophore has a fluorescent lifetime of at least 1 microsecond.

52. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore is BODIPY<sup>®</sup>-530/550 (4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-S-indacene).

53. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore is fluorescein.
54. (Canceled)
55. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore has an emissions maxima of about 603 nm.
56. (Canceled)
57. (Previously presented) The BoNT/A substrate of claim 4 or claim 53, wherein said acceptor fluorophore is tetramethylrhodamine.
58. (Previously presented) The BoNT/A substrate of claim 4 or claim 55, wherein said acceptor fluorophore has an excitation maxima of about 679 nm.
59. (Previously presented) The BoNT/A substrate of claim 4 or claim 52, wherein said acceptor fluorophore is BODIPY<sup>®</sup>-542/563 (4,4 difluoro-5-p-methoxyphenyl-4-bora-3a,4a-diaza-S-indacene).
60. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore is BODIPY<sup>®</sup>-542/563 (4,4 difluoro-5-p-methoxyphenyl-4-bora-3a,4a-diaza-S-indacene).
61. (Previously presented) The BoNT/A substrate of claim 4 or claim 60, wherein said acceptor fluorophore is BODIPY<sup>®</sup>-564/570 (4,4 difluoro-5-styryl-4-bora-3a,4a-diaza-S-indacene).
62. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore is Cy3.

63. (Previously presented) The BoNT/A substrate of claim 4 or claim 62, wherein said acceptor fluorophore is Cy5.

64-95. (Canceled)

96. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 20 residues.

97. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 40 residues.

98. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 50 residues.

99. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 100 residues.

100. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 150 residues.

101. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 200 residues.

102. (Previously presented) A botulinum toxin serotype A (BoNT/A) substrate, comprising:

(a) a donor fluorophore;

(b) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

- (c) a BoNT/A recognition sequence comprising a cleavage site, said BoNT/A recognition sequence comprising the amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof;

wherein said cleavage site intervenes between said donor fluorophore and said acceptor;

wherein said donor fluorophore, said acceptor, or both said donor fluorophore and said acceptor is not positioned within amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof; and

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor.

103. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 29, or a peptidomimetic thereof.
104. (Previously presented) The substrate of claim 102, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 30, or a peptidomimetic thereof.
105. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 1 nanomole/minute/milligram toxin.
106. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 20 nanomole/minute/milligram toxin.
107. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 50 nanomole/minute/milligram toxin.

108. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 100 nanomole/minute/milligram toxin.
109. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 150 nanomole/minute/milligram toxin.
110. (Previously presented) The BoNT/A substrate of claim 102, wherein said acceptor is an acceptor fluorophore.
111. (Previously presented) The BoNT/A substrate of claim 110, wherein said acceptor fluorophore has a fluorescent lifetime of at least 1 microsecond.
112. (Previously presented) The BoNT/A substrate of claim 102, wherein said acceptor is a non-fluorescent acceptor.
113. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is fluorescein.
114. (Currently amended) The BoNT/A substrate of claim 102, wherein said donor fluorophore is EDANS.
115. (Previously presented) The BoNT/A substrate of claim 102 or 113, wherein said acceptor is a fluorophore, said acceptor fluorophore being tetramethylrhodamine.
116. (Currently amended) The BoNT/A substrate of claim 102 or 114, wherein said acceptor is a non-fluorescent acceptor, said non-fluorescent acceptor being ~~DANCYL~~ DABCYL.
117. (Currently amended) The BoNT/A substrate of claim 112, wherein said non-fluorescent acceptor is DNP, DABCYL, or DABSYL ~~or QSY<sup>®</sup>-7~~.

118. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate has at most 100 residues.
119. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate has at most 50 residues.
120. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate has at most 40 residues.
121. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate has at most 20 residues.
122. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most fifteen residues.
- 123-125. (Canceled)
126. (Previously presented) A botulinum toxin serotype A (BoNT/A) substrate, comprising:
- (a) a donor fluorophore;
  - (b) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and
  - (c) a BoNT/A recognition sequence comprising a cleavage site, said BoNT/A recognition sequence comprising SEQ ID NO: 29, or a peptidomimetic thereof;
- wherein said cleavage site intervenes between said donor fluorophore and said acceptor;
- wherein said donor fluorophore or said acceptor is genetically encoded; and

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor.

127. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore is genetically encoded.
128. (Previously presented) The BoNT/A substrate of claim 126, wherein said acceptor is genetically encoded.
129. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are genetically encoded.
130. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate comprises at least six consecutive residues of SNAP-25, said six consecutive residues comprising Gln-Arg.
131. (Previously presented) The BoNT/A substrate of claim 130, wherein said BoNT/A substrate comprises at least six consecutive residues of a human SNAP-25, said six consecutive residues comprising Gln<sub>197</sub>-Arg<sub>198</sub>.
132. (Previously presented) The BoNT/A substrate of claim 131, wherein said BoNT/A substrate comprises the amino acid sequence Glu-Ala-Asn-Gln-Arg-Ala-Thr-Lys (SEQ ID NO: 1).
133. (Previously presented) The BoNT/A substrate of claim 131, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 27.
134. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129, wherein said BoNT/A substrate can be cleaved with an activity of at least 1 nanomole/minute/milligram toxin.



135. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129 wherein said BoNT/A substrate can be cleaved with an activity of at least 20 nanomole/minute/milligram toxin.
136. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129, wherein said BoNT/A substrate can be cleaved with an activity of at least 50 nanomole/minute/milligram toxin.
137. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129, wherein said BoNT/A substrate can be cleaved with an activity of at least 100 nanomole/minute/milligram toxin.
138. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129, wherein said BoNT/A substrate can be cleaved with an activity of at least 150 nanomole/minute/milligram toxin.
139. (Previously presented) The BoNT/A substrate of claim 126, wherein said acceptor is an acceptor fluorophore.
140. (Previously presented) The BoNT/A substrate of claim 139, wherein said acceptor fluorophore has a fluorescent lifetime of at least 1 microsecond.
141. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at most 400 residues.
142. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at most 500 residues.
143. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at most 600 residues.

144. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at most 700 residues.
145. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most fifteen residues.
146. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most thirty-five residues.
- 147-148. (Canceled)
149. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most ten residues.
150. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most fifteen residues.
151. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most twenty residues.
152. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most thirty residues.
153. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most forty residues.
154. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate is selected from the group consisting of SEQ ID NO: 85, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94 and SEQ ID NO: 95.

155. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most twenty residues.
156. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most twenty-five residues.
157. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most thirty residues.
158. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most thirty-five residues.
159. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most forty residues.
160. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 300 residues.
161. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 400 residues.
162. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 500 residues.
163. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 600 residues.
164. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 700 residues.
165. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most twenty residues.

166. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most twenty-five residues.
167. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most thirty residues.
168. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most forty residues.
169. (Canceled).
170. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 50 residues.
171. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 75 residues.
172. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 100 residues.
173. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 125 residues.
174. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 150 residues.
175. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 200 residues.
176. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most ten residues.

177. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most eight residues.
178. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most six residues.
179. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is not positioned within amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof.
180. (Previously presented) The BoNT/A substrate of claim 102, wherein said acceptor is not positioned within amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof.
181. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are not positioned within amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof.
182. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate comprises at least six consecutive residues of SNAP-25, said six consecutive residues comprising Gln-Arg.
183. (Previously presented) The BoNT/A substrate of claim 182, wherein said BoNT/A substrate comprises at least six consecutive residues of a human SNAP-25, said six consecutive residues comprising Gln<sub>197</sub>-Arg<sub>198</sub>.
184. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is BODIPY<sup>®</sup>-530/550 (4,4 difluoro 5,7 diphenyl 4 bora-3a,4a-diaza-S-indacene).

185. (Previously presented) The BoNT/A substrate of claim 102 or claim 184, wherein said acceptor is a fluorophore, said acceptor fluorophore being BODIPY<sup>®</sup>-542/563 (4,4 difluoro-5-p-methoxyphenyl-4-bora-3a,4a-diaza-S-indacene).
186. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is BODIPY<sup>®</sup>-542/563 (4,4 difluoro-5-p-methoxyphenyl-4-bora-3a,4a-diaza-S-indacene).
187. (Previously presented) The BoNT/A substrate of claim 102 or claim 186, wherein said acceptor is a fluorophore, said acceptor fluorophore being BODIPY<sup>®</sup>-564/570 (4,4 difluoro-5-styryl-4-bora-3a,4a-diaza-S-indacene).
188. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is Cy3.
189. (Previously presented) The BoNT/A substrate of claim 102 or claim 188, wherein said acceptor is a fluorophore, said acceptor fluorophore being Cy5.
190. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore has an emission maxima of about 603 nm.
191. (Previously presented) The BoNT/A substrate of claim 102 or claim 190, wherein said acceptor is a fluorophore, said acceptor fluorophore having an excitation maxima having an excitation maxima of about 679 nm.
192. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore has an emission maxima of about 690 nm.
193. (Previously presented) The BoNT/A substrate of claim 102 or claim 192, wherein said acceptor is a fluorophore, said acceptor fluorophore having an excitation maxima of about 749 nm.

194. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is pyrene.
195. (Previously presented) The BoNT/A substrate of claim 102 or claim 194, wherein said acceptor is a fluorophore, said acceptor fluorophore being coumarin.
196. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate comprises at most 300 residues.
197. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate comprises at most 350 residues.
198. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A recognition sequence comprises amino acids 137 to 206 of SEQ ID NO: 2.
199. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A recognition sequence comprises amino acids 134 to 206 of SEQ ID NO: 2.
200. (Previously presented) The BoNT/A substrate of either claim 127 or 129, wherein said genetically encoded donor fluorophore is selected from the group consisting of a blue fluorescent protein, a cyan fluorescent protein, a green fluorescent protein, a yellow fluorescent protein and a red fluorescent protein.
201. (Previously presented) The BoNT/A substrate of either claim 128 or 129, wherein said genetically encoded acceptor is a fluorophore, said genetically encoded acceptor fluorophore selected from the group consisting of a blue fluorescent protein, a cyan fluorescent protein, a green fluorescent protein, a yellow fluorescent protein and a red fluorescent protein.
202. (Previously presented) The BoNT/A substrate of claim 129, wherein said genetically-encoded acceptor is a fluorophore.

203. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a blue fluorescent protein, said acceptor fluorophore is a green fluorescent protein and said BoNT/A recognition sequence comprises SEQ ID NO: 29.
204. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a blue fluorescent protein, said acceptor fluorophore is a green fluorescent protein and said BoNT/A recognition sequence comprises amino acids 137-206 of SEQ ID NO: 2.
205. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a blue fluorescent protein, said acceptor fluorophore is a green fluorescent protein and said BoNT/A recognition sequence comprises amino acids 134-206 of SEQ ID NO: 2.
206. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a blue fluorescent protein, said acceptor fluorophore is a green fluorescent protein and said BoNT/A recognition sequence comprises SEQ ID NO: 2.
207. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a green fluorescent protein, said acceptor fluorophore is a red fluorescent protein and said BoNT/A recognition sequence comprises SEQ ID NO: 29.
208. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a cyan fluorescent protein, said acceptor fluorophore is a yellow fluorescent protein and said BoNT/A recognition sequence comprises SEQ ID NO: 29.
209. (Previously presented) The BoNT/A substrate of claim 127, wherein said acceptor is a fluorophore.
210. (Previously presented) The BoNT/A substrate of claim 209, wherein said donor fluorophore is a green fluorescent protein, said acceptor fluorophore has an excitation



maxima of about 556 nm and said BoNT/A recognition sequence comprises SEQ ID NO: 29.

211. (Previously presented) The BoNT/A substrate of claim 209, wherein said donor fluorophore is a red fluorescent protein, said acceptor fluorophore has an excitation maxima of about 632 nm and said BoNT/A recognition sequence comprises SEQ ID NO: 29.